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# MALARIA CONTROL UTILIZING *BACILLUS SPHAERICUS* AGAINST *ANOPHELES STEPHENSI* IN PANAJI, GOA

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ABSTRACT. In a large malaria endemic area in Panaji city, Goa, India, the weekly application of the biolarvicide *Bacillus sphaericus* (Strain 101, Serotype H 5a 5b) at the rate of 1 g/m$^2$ in the main *Anopheles stephensi* larval habitats, *viz.*, curing waters, masonry tanks, and sump tanks (under construction), from April to December 1993 resulted in a sharp decline in the habitat positivity (range 0.13–8.0%) as compared with the rest of the Panaji (range 2.2–30.6%) where temephos (Abate) was used as the larvicide. *Bacillus sphaericus* spraying also led to a significant decline in anopheline densities in positive habitats (range 0–7.3/10 dips) as compared with control habitats (range 0.9–53.0/10 dips). Concurrently, malaria incidence observed in the experimental area (slide positivity rate [SPR] range 2.3–7.8%; monthly parasite index [MPI] range 0.18–1.44) was lower than in the control area (SPR range 14.3–25.5%; MPI range 1.75–6.12).

## INTRODUCTION

Drawbacks associated with insecticidal use, such as vector resistance to these compounds, environmental pollution, and costs, have led to the search for alternatives that meet with the constraints imposed by the vector control programs. Among the alternatives, biological control agents, larvivorous fishes, and many strains of spore-forming bacteria (*Bacillus sphaericus* and *Bacillus thuringiensis* H-14) have proved their usefulness for vector control in many field trials (Des Rochers and Garcia 1984, Mulla et al. 1984, Mittal et al. 1985). Although *B. sphaericus* was described by Neide in 1904, the first evidence of its insecticidal properties was provided by Kellen and Meyers (1964), who isolated this bacteria from moribund larvae of *Culiseta incidens* (Thomson) in California, USA. Since then, a large number of atoxic and toxic strains of *B. sphaericus* have been discovered. The toxic strains have been serotyped and grouped according to the degree of their efficacy (Singer 1990). The strains highly toxic to mosquito larvae, such as 1593, 1593-4, 1691, 1881, and 2362, fall in serotype group H 5a 5b (de Barjac 1990). Mittal et al. (1985) evaluated HIL 9 and HIL 10 formulations of *B. sphaericus* and reported 100% mortality in 3rd- and 4th-instar larvae of *Anopheles culicifacies* Giles, which is the principal rural malaria vector in India. In another study, Solvay liquid 2362 and granular 2297 formulations of *B. sphaericus* were effective against *An. culicifacies* and *Anopheles stephensi* Liston for 1 week (Ansari et al. 1989).

In the post-resurgence phase after 1977, Goa State in India remained hypoendemic to malaria with the annual parasite incidence (API) below 2 until a major outbreak occurred in the capital city of Panaji in 1986 with 352 cases reported (API 8.2). The malaria incidence increased sharply in 1987 (cases 4,406, API 102.4) and 1988 (cases 5,677, API 132.0) before showing a significant decline with the introduction of bioenvironmental measures starting in 1989. The APIs reported were 82.0 in 1989, 88.0 in 1990, 36.2 in 1991, and 10.4 in 1992. After the bioenvironmental control demonstration project ended in 1992, the incidence once again spurted in 1993 with an API of 32.8.

Vector interventions from 1986 onwards included 4% DDT house spraying, thermal fogging with pyrethrum, and Abate® application at 1 ppm dose aimed at *An. stephensi* larval control. The bioenvironmental supplementary measures from 1989 to 1992 included extensive use of larvivorous fishes (*Aplocheilus blocki, Gambusia affinis, Poecilia reticulata, Oreochromis mossambicus,* and *Rasbora daniconius*) in wells, masonry tanks, underground tanks and larger bodies of water in the basements of buildings under construction. Additional larval control measures included adding expanded polystyrene beads to disused wells, habitat drying with suction pumps, and seiving of larvae with micro-nets from exposed overhead cisterns and sumps. Selective spraying of *B. thuringiensis* and *B. sphaericus* was also done in stagnant curing water on newly cast roofs in the construction sites.

In Panaji city, prolific breeding of *An. stephensi* in construction sites together with aggregation of migratory labor in their vicinity played a major role in the epidemic of malaria that broke out in 1986 (Kumar et al. 1991). A large-scale field study was undertaken in Panaji during 1993 using the

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B 101 (Spherix) formulation of *B. sphaericus* to evaluate the impact of this biocontrol agent on *An. stephensi* and the incidence of malaria. The results of the study are discussed in this paper.

## MATERIALS AND METHODS

The study was conducted in the malaria endemic localities of Miramar-Tonca in Panaji city in an area of 2 $km^2$ beginning in April 1993. The major breeding sites of *An. stephensi* (masonry tanks, sump tanks, and curing waters on concrete slabs and freshly constructed overhead tanks) were treated with Spherix[4] at the rate of 1 $g/m^2$ surface area with knapsack sprayers with flat fan nozzles.

The *B. sphaericus* formulation was homogeneous with particle sizes 90–500 $\mu m$, bulk density 0.49–0.58 $g/cm^2$, and pH 6.05–6.4. The $LD_{50}$ values of the product ranged from 0.008 to 0.012 mg/liter against *Culex pipiens* Linn. (autogenous strain), and potency was 450 ITU/mg against the SPH 8 standard reference powder. Both endotoxins and spores constitute 14–20% dry weight of the formulation.

Before the starting of Spherix spray operations, all the chemical control methods (DDT spraying, thermal fogging, and temephos spraying) were withdrawn from the experimental area by the State Health Services. Wells in both experimental and control areas contained larvivorous fishes. In the experimental area, all habitats, irrespective of their breeding status, initially were sprayed with Spherix. Subsequent weekly application was done only in positive habitats detected during larval surveillance. For the estimation of immature densities, breeding habitats were sampled weekly using a 300-ml bowl in the areas with maximum breeding, i.e., at the edges, corners, wooden logs, iron rods, or bricks immersed in water. The averages were calculated separately for different larval instars and pupae for the respective months. A similar weekly sampling of immatures of *An. stephensi* was done in the temephos-treated areas held as the control. The active case detection was done fortnightly by the National Malaria Eradication Program, Goa. Clinical laboratories at the Urban Health Centre, Panaji, Directorate of Health Services Headquarters, and Goa Medical College provided passive case detection and treatment facilities. For statistical analysis, student's $t$-test and $\chi^2$ for differences between the paired means were applied.

[4] The biolarvicide was manufactured by Berdsk Plant of Biological Preparation, Russia, and supplied under the trade name of Spherix through the courtesy of Ministry of Health and Family Welfare, Delhi, India.

## RESULTS AND DISCUSSION

*Impact of Spherix spraying on vector populations:* In Panaji, *An. stephensi* breeds abundantly during the monsoon season in the curing waters, masonry tanks, and sump tanks in construction sites (Kumar and Thavaselvam 1992). Monthly data on immatures of the vector collected during pre- and post-treatment phases from both experimental and control areas are presented in Table 1. Sharp reduction in habitat positivity and immature densities was observed in post-treatment months in the experimental area as compared with the control (Fig. 1). The impact was most pronounced during the monsoon months from May to July, which also happens to be the peak *Plasmodium* transmission period in Panaji (Kumar et al. 1991). Although the densities of *An. stephensi* immatures were low in both the experimental and control areas during the post-monsoon period from August to December, the differences in the habitat positivity ($t$ = 3.16, $P$ = 0.008, df = 7) and densities ($t$ = 2.06, $P$ = 0.04, df = 7) were statistically significant (Table 1).

*Impact on malaria incidence:* The parasitological data of both the experimental and control areas are shown in Table 2 and Fig. 2. In the post-treatment phase, i.e., May–December 1993, 40 cases of malaria were reported from the experimental area with the slide positivity rate (SPR) and the monthly parasite incidence (MPI) ranging from 2.3 to 7.8% and 0.18 to 4.4, respectively. There was no evidence of an increase in cases in the experimental area, but in the control area malaria incidence increased after May, with a peak of 237 cases reported during the month of July alone. Overall, 1,260 out of 1,360 total cases were reported in the control area from May to December 1993. In this area, the SPR and MPI during these months ranged from 14.3 to 25.5% and 1.75 to 6.12%, respectively. The difference in the incidence between the experimental and control area was found to be significant (MPI: $\chi^2$ = 2.08, $P$ = 0.04, df = 7) (Fig. 2).

It is clear that within a month of commencement of its spraying, the Spherix formulation at 1 $g/m^2$ brought about effective control of the vector in its major breeding habitats in the construction sites. It also was observed that a weekly treatment of only positive habitats kept vector breeding in check from June to December 1993. Consequently, the decline in habitat positivity and vector densities particularly during the monsoon season had the desired impact on malaria

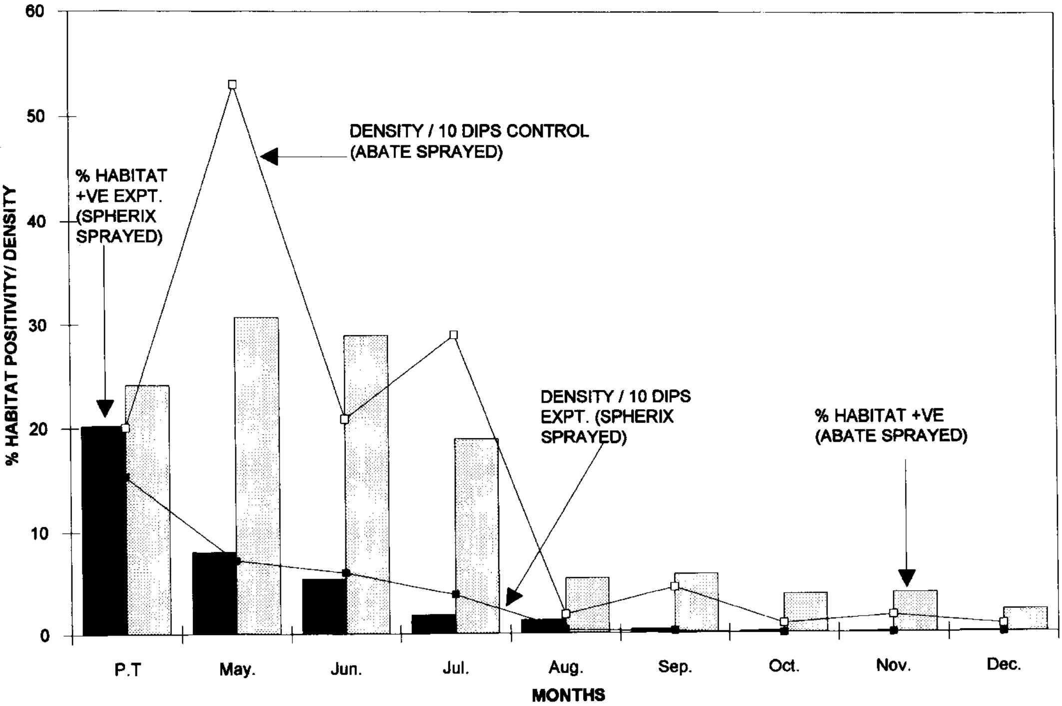


Fig. 1. Comparison of percentage of habitat positivity and density/10 dips of anophelines in construction sites between experimental area where *Bacillus sphaericus* (Spherix) was sprayed at 1 g/m² and control area in Panaji city. In the control area, breeding sites were treated with the chemical larvicide temephos (1 ppm) by the National Filaria Control Programme, Panaji Unit, Goa.

Table 1. Comparison of percent habitat positivity and densities of *Anopheles stephensi* in construction sites between the experimental area where *Bacillus sphaericus* (Spherix formulation) was sprayed at 1 g/m², and the control area in Panaji, Goa.

| Month 1993 | Percent habitat positive: Experimental (*n*) | Percent habitat positive: Control (*n*) | Densities/10 dips, Experimental (Spherix sprayed at 1 g/m²): Total dips | I + II | III + IV + pupa | Total | Densities/10 dips, Control (Abate sprayed at 1 ppm): Total dips | I + II | III + IV + pupa | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Pretreatment | | | | | | | | | | |
| April | 20.2 (99) | 24.1 (58) | 525 | 7.9 | 7.4 | 15.3 | 360 | 15.3 | 4.7 | 20.0 |
| Post-treatment | | | | | | | | | | |
| May | 8.0 (450) | 30.7 (62) | 2,855 | 4.5 | 2.8 | 7.2 | 360 | 30.7 | 22.3 | 53.0 |
| June | 5.4 (1,078) | 28.9 (97) | 6,230 | 2.4 | 3.6 | 6.0 | 605 | 14.0 | 6.8 | 20.8 |
| July | 1.8 (884) | 18.9 (106) | 5,180 | 2.3 | 1.5 | 3.8 | 640 | 18.5 | 10.4 | 28.9 |
| August | 1.3 (901) | 5.4 (205) | 5,585 | 0.1 | 0.1 | 0.3 | 1,110 | 0.4 | 1.4 | 1.8 |
| September | 0.4 (1,093) | 5.8 (206) | 5,740 | 0.2 | N | 0.2 | 1,090 | 2.2 | 2.3 | 4.5 |
| October | 0.2 (906) | 3.8 (185) | 5,475 | 0 | 0 | 0 | 980 | 0.3 | 0.6 | 0.9 |
| November | 0.1 (793) | 3.9 (130) | 4,745 | 0 | 0 | 0 | 705 | 0.5 | 1.2 | 1.7 |
| December | 0.1 (694) | 2.2 (135) | 4,290 | N[1] | 0 | N | 725 | 0.4 | 0.4 | 0.8 |

[1] N = negligible.

Table 2. Comparison of incidence of malaria in Spherix-sprayed area with rest of Panaji (Abate-treated area at 1 ppm).

| Month | Experimental area (pop. 5,530) | | | | | | | Control area (pop. 38,722) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1993 | B.S.[1] | Pos. | P.v. | P.f. | SPR | SFR | MPI | B.S.[1] | Pos. | P.v. | P.f. | SPR | SFR | MPI |
| Pre-treatment | | | | | | | | | | | | | | |
| January | 133 | 2 | 2 | 0 | 1.5 | 0 | 0.36 | 634 | 16 | 10 | 6 | 2.5 | 0.94 | 0.40 |
| February | 57 | 3 | 3 | 0 | 5.2 | 0 | 0.54 | 432 | 18 | 14 | 4 | 4.2 | 0.92 | 0.46 |
| March | 134 | 2 | 1 | 1 | 1.5 | 0.74 | 0.36 | 387 | 22 | 20 | 2 | 5.7 | 0.52 | 0.57 |
| April | 100 | 4 | 4 | 0 | 4 | 0 | 0.72 | 436 | 44 | 44 | 0 | 10.1 | 0 | 1.13 |
| Post-treatment | | | | | | | | | | | | | | |
| May | 194 | 7 | 6 | 1 | 3.6 | 0.50 | 1.26 | 427 | 68 | 65 | 3 | 15.9 | 0.7 | 1.75 |
| June | 260 | 6 | 6 | 0 | 2.3 | 0 | 1.08 | 937 | 135 | 122 | 13 | 14.4 | 1.4 | 3.48 |
| July | 347 | 8 | 8 | 0 | 2.3 | 0 | 1.44 | 1,506 | 237 | 190 | 47 | 15.7 | 3.1 | 6.12 |
| August | 103 | 4 | 4 | 0 | 3.9 | 0 | 0.72 | 1,433 | 205 | 174 | 31 | 14.3 | 2.2 | 5.29 |
| September | 85 | 6 | 5 | 1 | 7 | 1.17 | 1.08 | 940 | 155 | 115 | 40 | 16.5 | 4.25 | 4.00 |
| October | 55 | 4 | 4 | 0 | 7.3 | 0 | 0.72 | 1,042 | 215 | 136 | 79 | 20.6 | 7.6 | 5.55 |
| November | 41 | 1 | 1 | 0 | 2.4 | 0 | 0.18 | 669 | 138 | 91 | 47 | 20.6 | 7.0 | 3.56 |
| December | 51 | 4 | 4 | 0 | 7.8 | 0 | 0.72 | 419 | 107 | 68 | 39 | 25.5 | 9.3 | 2.76 |
| Total | 1,560 | 51 | 48 | 3 | 3.27 | 0.19 | 9.22 | 9,262 | 1,360 | 1,049 | 311 | 14.68 | 3.35 | 35.12 |

Note: Blood slide collection was done and treatment of malaria cases was given by NMEP, Goa, in both areas.

[1] B.S. = Blood slides examined, Pos. = No. positive, P.v. = *Plasmodium vivax*, P.f. = *Plasmodium falciparum*, SPR = slide positivity rate, SFR = slide falciparum rate, MPI = monthly parasite incidence (per 1,000).

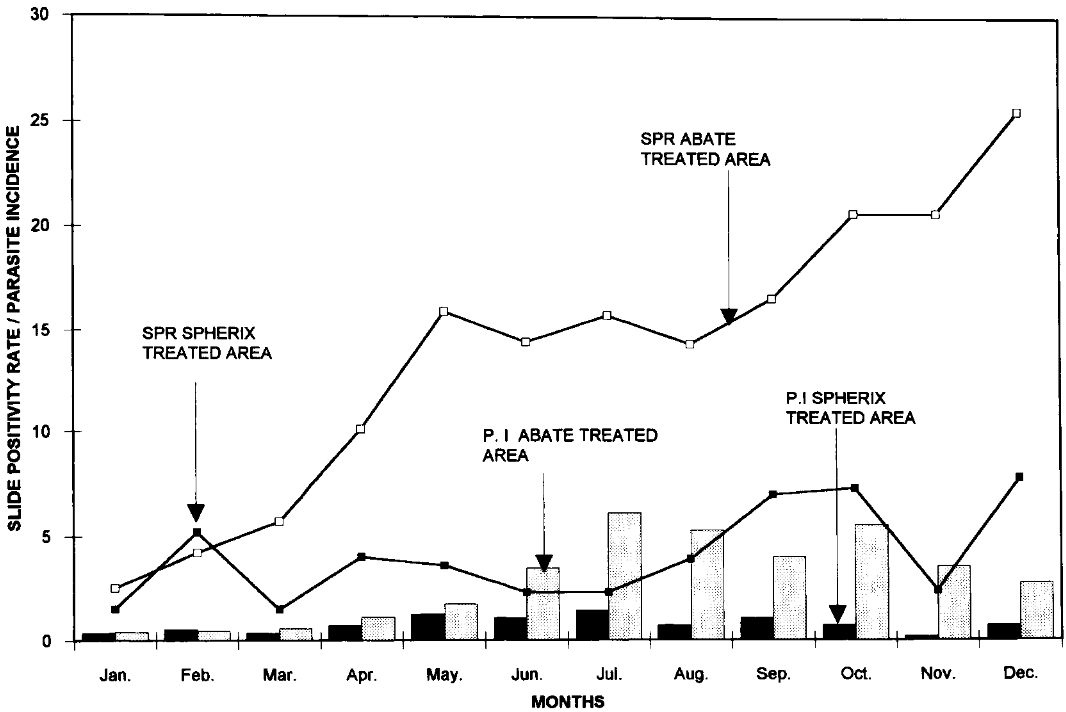


Fig. 2. Comparison of slide positivity rate (SPR) and monthly parasite incidence (MPI) between Spherix-sprayed area and the temephos-sprayed control area.

incidence, which remained low even during the peak transmission months of July and August. But in the untreated control area, there was a much greater vector output, particularly from May to July, which resulted in very active malaria transmission not only during monsoon months but also in the post-monsoon period when the vector potential in this area gradually declines (Kumar and Thavaselvam 1992). The increased parasite load in the population during the monsoon period in the control area seemed to have sustained transmission in the post-monsoon months.

Recent larval bioassay studies have shown that *An. stephensi* larval populations in Panaji and surroundings are fully susceptible to temephos (Abate) at the 1-ppm recommended dose, although adults show 87% resistance against 4% DDT (Thavaselvam et al. 1993). The failure of temephos to control breeding in the epidemiologically important construction sites appears to be due to inadequate coverage and that of DDT spraying due to a high degree of resistance coupled with high refusal rate for house spraying.

In conclusion, the *B. sphaericus* strain B 101 serotype H 5a 5b used in this longitudinal field study has proved to be a useful biocontrol agent for *An. stephensi*. Bhalwar et al. (1993) have also reported the usefulness of this formulation in the effective control of the filaria vector *Culex quinquefasciatus* Say. Economically, both material and operational costs for vector control with *B. sphaericus* and *B. thuringiensis* have been found to be comparable with fenthion and significantly less than larvicidal oils, temephos, and Paris green. Their commercial availability will strengthen the use of alternate methods of vector control, the search for which has become necessary due to a number of practical constraints posed by chemical insecticides.

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